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Original Contribution

Cancer Incidence among Male Pesticide Applicators in the Agricultural Health Study Cohort Exposed to Diazinon

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Little is known about the potential carcinogenicity associated with routine application of diazinon, a common organophosphate insecticide. The authors explored a possible association of diazinon exposure with cancer risk in the Agricultural Health Study, a prospective cohort of licensed pesticide applicators in Iowa and North Carolina enrolled in 1993–1997. A total of 23,106 male applicators provided information in a self-administered questionnaire. Among 4,961 applicators who reported using diazinon, 301 incident cancer cases were diagnosed during the follow-up period ending December 2002 compared with 968 cases among 18,145 participants who reported no use. Poisson regression was used to calculate rate ratios and 95% confidence intervals. Two quantitative exposure metrics were used: lifetime exposure days and intensity-weighted lifetime exposure days, a measure that incorporates probability of pesticide exposure with lifetime pesticide application frequency. When lifetime exposure days were used, increased risks for the highest tertile of exposure and significant tests for trend for lung cancer and leukemia were observed. No other cancer site showed an association with diazinon for the highest tertile of exposure. Because these results were based on small numbers, additional analyses are necessary as more cases accrue to clarify whether diazinon is associated with cancer risk in humans.

cohort studies; diazinon; insecticides; neoplasms; pesticides

Abbreviations: CI, confidence interval; EPA, Environmental Protection Agency; RR, rate ratio.

Diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] is a common organophosphate insecticide registered for a variety of uses on plants and animals. Approximately 4 million pounds of diazinon were applied in agricultural settings in the United States in 2004 (1–3). Diazinon is registered for use on fruit, nut, and ornamental crops, as well as in cattle ear tags, and has been available in a variety of formulations, including dust, granules, seed dressings, wettable powders, emulsifiable-solution formulations, and impregnated pet collars and pest strips (4). Historically, it was commonly used in household insecticide products (3). In 2001, it was the most common active ingredient in

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insecticides in the home and garden market (5) even though the US Environmental Protection Agency (EPA) started phasing out all residential product registrations for diazinon in 2000. Complete phase-out for the home market was completed in December 2004 because of noncancer health risks (6). The EPA has also proposed new restrictions on agricultural use to protect workers and the environment (3).

The standard assays of mutagenicity and cytotoxicity for diazinon have generally been negative (7, 8). The EPA reviewed the data on carcinogenicity of the pesticide in 1997 and classified it as ''not likely a human carcinogen'' because of the lack of carcinogenicity in mice and rats. However, some laboratory and epidemiologic data suggest potential carcinogenicity. In rodent feeding studies, diazinon has been shown to be associated with organ and tissue abnormalities, which may have resulted from oxidative stress due to diazinon metabolism (9, 10). Sister chromatid exchange induction, a marker of chromosomal damage, has been shown in some in vitro studies of diazinon (11, 12) but not in others (13–16). In an in vitro study of human nasal mucosal cells, researchers observed a dose-response genotoxic effect measured by comet assay (17). Human in vivo studies have shown a variety of effects; in one study, sister chromatid exchange was elevated in human peripheral blood lymphocytes after occupational exposure to diazinon (12). There is some suggestion that carcinogenesis from diazinon exposure could occur from either decreased immunosurveillance or direct malignant transformation (9).

Two case-control studies have shown an increased risk of non-Hodgkin's lymphoma with exposure to diazinon (18, 19). Another study that combined results from previous case-control studies of non-Hodgkin's lymphoma showed not only an association between diazinon use and non-Hodgkin's lymphoma but also effect modification of the association by other pesticides, such as atrazine (20). Parental diazinon use was linked to childhood brain cancer in a case-control study (21). An association between diazinon use and lung cancer was reported in an earlier analysis of the Agricultural Health Study cohort (22) and in a cohort of pesticide applicators in Florida (23). Although some evidence exists that diazinon is related to an increased risk of some cancers, there is a dearth of evidence about the possible association with many others. The International Agency for Research on Cancer has not reviewed the carcinogenicity of diazinon in particular; however, the agency does state that spraying and application of nonarsenical insecticides falls in the category of group 2A, or ''probably carcinogenic to humans'' (24), indicating that further investigation into this class of chemicals is warranted. Here, we explore the potential association of exposure to diazinon with an increased risk of cancer in the Agricultural Health Study.

MATERIALS AND METHODS

Cohort enrollment and follow-up

The Agricultural Health Study is a prospective cohort study of 57,311 licensed pesticide applicators and their spouses in Iowa and North Carolina (25). In North Carolina, only those applicators who were farmers were recruited; in Iowa, both commercial and farm applicators were included. Commercial applicators included those persons employed by pest control companies or businesses that use pesticides. Applicators were recruited from December 1993 through December 1997 from mandatory certification sessions for using EPA-designated Restricted Use Pesticides. At the certification session, participants completed an enrollment questionnaire and were given a take-home questionnaire that sought more detailed information about a variety of exposures.

To enable identification of incident cancer cases, information on cohort members was matched to cancer registry files in Iowa and North Carolina. For this study, all incident cancer cases diagnosed through December 31, 2002, were included (Agricultural Health Study data release version P1REL0502). Annually, cohort members' data were matched to the National Death Index to identify vital status and to current address records of the Internal Revenue Service, motor vehicle registration offices, and pesticide license registries of state agricultural departments to identify whether the participants continued to reside in Iowa or North Carolina. Follow-up was censored at the time of participant death or movement out of the state. All participants provided informed consent, and the protocol was approved by the institutional review boards of the National Cancer Institute, Battelle, the University of Iowa, and Westat (Rockville, Maryland).

Exposure assessment

Exposure to diazinon and other factors was assessed through the completion of enrollment and take-home, self-administered questionnaires. The questionnaires are available online at http://www.aghealth.org/questionnaires.html. In the enrollment questionnaire, participants provided information on ever/never use of 50 commonly used pesticides as well as detailed information on lifetime exposure (number of days per year and number of years) for 22 pesticides, pesticide application and mixing methods, the repair of equipment, and the use of personal protective equipment. They also reported on potential risk factors such as smoking, alcohol consumption, cancer history of first-degree relatives, diet, selected medical conditions, and demographic information. In the take-home questionnaire, participants provided lifetime exposure information on the remaining 28 pesticides, including diazinon.

In addition to lifetime exposure days (number of application days per year times number of years of application), a pesticide exposure intensity score was calculated. This intensity score was based on the frequency and duration of application, application method, mixing and equipment repair status, and use of personal protective equipment. These factors were weighted to reflect the intensity of exposure based on monitoring data from the literature. The following algorithm was used to calculate the exposure intensity: ([application method + mixing status + equipment repair status] $\times$ personal protective equipment use) (26).

Data analysis

Detailed information on the use of diazinon was collected from the take-home questionnaire; therefore, the current analyses were restricted to those participants who completed this questionnaire ($n = 25,291$). The characteristics of farmers who completed only the enrollment questionnaire were similar to those of farmers who also completed and returned the take-home questionnaire (27). A total of 953 applicators were excluded because they provided no information on diazinon use. Analyses excluded female applicators ($n = 654$) since there were only 187 women who reported ever being exposed to diazinon and too few cases to perform separate analyses. Finally, to ensure that exposure assessment preceded the development of cancer, all analyses excluded applicators who had a cancer diagnosis prior to enrolling in the study ($n = 578$). After these exclusions, data on 23,106 applicators were included in these analyses.

We evaluated diazinon exposure by using three methods: ever or never use, lifetime exposure days, and intensity-weighted lifetime exposure days. In the latter two instances, exposure was categorized into tertiles based on the exposure distribution of all cancer cases. In this paper, results are reported for cancer sites for which there were more than 10 exposed cases based on lifetime days of exposure. To explore the consistency of observed associations between Iowa and North Carolina, we stratified analyses and formally tested for effect modification by state.

Poisson regression analysis was performed by using the Stata statistical software program (release 8.0; Stata Corporation, College Station, Texas) to calculate rate ratios. All rate ratios were adjusted for age as a categorical variable (<40, 40–49, 50–59, 60–69, 70–79, ≥80), smoking history (never, former smoker <3.75 pack-years, former smoker 3.75–15 pack-years, former smoker >15 pack-years, current smoker <11.5 pack-years, current smoker 11.5–28.4 pack-years, current smoker ≥28.5 pack-years), alcohol consumption (never/ever in past year), education (high school or less, greater than high school), state of residence (Iowa, North Carolina), family history of cancer, and lifetime days of any pesticide application (continuous variable based on days and years of use). All analyses were also performed by using two different referent groups: those reporting no use of diazinon and those in the lowest tertile of diazinon use. The two referent groups were used because uncontrolled confounding could occur if the nonexposed group was different from the high exposed group regarding unmeasured (and therefore uncontrollable) risk factors. We used two quantitative exposure metrics and performed a test for linear trend by using the median of each exposure category as the quantitative score. All tests for significance were two sided.

RESULTS

Of the 23,106 eligible applicators who completed the take-home questionnaire, 4,961 (21.5 percent) reported ever using diazinon. Among diazinon users, the mean number of years that diazinon was applied was 6.1 (standard deviation, 5.1) and the mean number of days per year was 7.7 (standard deviation, 15.2). There were 301 incident cancer cases diagnosed during the follow-up period in diazinon users compared with 968 cases of incident cancer among 18,145 nonexposed study participants who completed the take-home questionnaire. The adjusted rate ratio for all incident cancers for those exposed compared with those nonexposed was 1.16 (95 percent confidence interval (CI): 1.00, 1.35).

Table 1 shows the distribution of selected demographic characteristics among three diazinon exposure groups: nonexposed, lowest exposed tertile, and highest two tertiles of lifetime exposure days based on the distribution of all cancer cases. A total of 4,809 (97 percent) of the 4,961 participants who reported ever using diazinon provided detailed information about their use. The component parts of lifetime days, days per year, and total years applied were assessed through categories. Therefore, when the categories were multiplied, many participants had the same number of exposure days, which led to an unequal distribution in the tertile categories. Regardless of diazinon exposure, approximately 65 percent of participants were never smokers or very-low-exposure former smokers (<3.75 pack-years). Those most highly exposed to diazinon also reported more total days of any pesticide application. The three exposure groups (none, low, and high) were similar with respect to most demographic characteristics. However, the higher exposed were more similar to the low exposed than the nonexposed on a few measures, such as state of residence and education, which could have led to residual confounding from factors not measured.

Tables 2 and 3 show the results of analyses exploring the association of diazinon use with selected cancers. For lifetime exposure days, we found increased risks for the highest exposure category compared with the nonexposed category regarding the incidence of all cancers (rate ratio (RR) = 1.39, 95 percent CI: 1.09, 1.78), lung cancer (RR = 2.41, 95 percent CI: 1.31, 4.43), and leukemia (RR = 3.36, 95 percent CI: 1.08, 10.49). We also observed a significant increasing linear trend for the incidence of these cancers (p-trend for all cancers = 0.009, p-trend for lung cancer = 0.005, p-trend for leukemia = 0.026). When the low-exposed tertile was used as the referent group, the association with only lung cancer remained significant (RR = 3.19, 95 percent CI: 1.28, 7.93), although the association with all cancers approached significance (RR = 1.34, 95 percent CI: 0.99, 1.82). We found results similar to those overall when we stratified by state of residence (data not shown).

The elevated risks observed with lifetime exposure days for all cancers, lung cancer, and leukemia were somewhat attenuated when we used intensity-weighted lifetime days as the exposure metric (table 3). Of the sites for which we observed an elevated association with lifetime exposure days, only that for all cancer sites remained significantly elevated. Although the point estimates for lung cancer and leukemia were elevated, the confidence intervals included the null. We observed an elevated risk estimate and test for trend for all lymphohematopoietic cancers with the highest tertile of intensity-weighted exposure when we used the

TABLE 1. Selected characteristics of male diazinon applicators, by exposure category, based on 1993–1997 enrollment data, Agricultural Health Study*

Characteristic	Exposure category					
	Nonexposed ($n = 18,145$)	%	Lowest exposed ($n = 2,158$)	%	Highest exposed ($n = 2,651$)	%
Age (years)						
<40	5,183	28.6	503	23.3	568	21.4
40–49	4,860	26.8	619	28.7	708	26.7
50–59	3,889	21.4	520	24.1	683	25.8
≥60	4,213	23.2	516	23.9	692	26.1
State of residence						
Iowa	13,566	74.8	1,242	57.6	1,168	44.1
North Carolina	4,579	24.2	916	42.4	1,483	55.9
Type of applicator						
Private	16,532	91.1	1,975	91.5	2,262	85.3
Commercial	1,613	8.9	183	8.5	389	14.7
Smoking history (pack-years)†						
Never	9,740	56.4	1,087	53.0	1,119	45.1
Former	5,194	33.0	689	33.6	911	36.7
Current	2,342	13.6	275	13.4	452	18.2
Alcohol consumption†						
Never in the last year	5,516	31.9	715	34.5	953	38.1
Ever in the past year	11,803	68.1	1,356	65.5	1,549	61.9
Education†						
High school or less	10,871	60.0	1,041	48.3	1,325	50.1
Greater than high school	7,237	40.0	1,115	51.7	1,318	49.9
Family history of cancer†						
No	9,613	58.4	1,055	53.0	1,263	53.7
Yes	6,863	41.6	934	47.0	1,088	46.3
Mean (standard deviation) lifetime no. of days of all pesticide application	342.3 (539.3)		357.8 (491.9)		638.5 (860.2)	
Mean (standard deviation) years of follow-up	7.43 (1.54)		7.47 (1.56)		7.43 (1.64)	

* Restricted to those without prior cancer and those who completed a take-home questionnaire.
† Values do not equal the total because of missing data.

nonexposed group as the reference. When the low-exposure group was used as the reference category, none of the risks were significantly elevated.

To further explore associations that might exist at higher levels of exposure, we performed analyses that split the highest tertile of exposure at the median of that tertile (table 4). We conducted these analyses for both lifetime and intensity-weighted exposure and here report only those results for cancers that affected more than 25 exposed cases (all sites, lung, prostate, and lymphohematopoietic). For lifetime exposure days, we observed an increasing monotonic trend and significant associations for all cancer sites when using either reference group. For lung cancer, the highest level of exposure corresponded to a rate ratio of 3.46 (95 percent CI: 1.57, 7.65) for lifetime exposure days when the nonexposed group was used as the referent, and the rate ratio was 4.16 (95 percent CI = 1.47, 11.81) when the low-exposed group was the referent. When the intensity-weighting algorithm was used, the rate ratio for all cancer sites remained elevated, but only when we used the nonexposed group as the referent. Additionally, lymphohematopoietic cancer risk was elevated in the most highly exposed group. When we examined all cancer sites, excluding lung, prostate, and lymphohematopoietic, we saw a similar pattern of increasing rate ratio with increasing exposure, but the results were not statistically significant (data not shown).

In addition to the models that controlled for exposure to other pesticides by using total lifetime days of any pesticide exposure, we also modeled cancer risk by using the five pesticides most highly correlated with diazinon (ethylene dibromide, aluminum phosphide, metalaxyl, chlordane, and dieldrin) as covariates. The resulting risk estimates were not markedly different from the results presented here. A previous report from the Agricultural Health Study showed an increased risk of lymphohematopoietic cancers

TABLE 2. Rate ratios for selected cancers through December 2002, by lifetime exposure days to diazinon, in male Agricultural Health Study pesticide applicators

Cancer site (ICD-9* classification)	Lifetime no. of exposure days	No. of cases	Referent: nonexposed category		Referent: low-exposed category	
			RR*,†	95% CI*	RR†	95% CI
All neoplasms (codes 140–208)	No exposure	722	1.0			
	<20	106	1.12	0.91, 1.38	1.0	
	20.0–38.8	64	1.08	0.83, 1.40	1.00	0.73, 1.37
	>38.8	77	1.39	1.09, 1.78	1.34	0.99, 1.82
			p-trend = 0.009		*p*-trend = 0.043	
Colorectal (codes 153 and 154)	No exposure	57	1.0			
	<20	6	0.92	0.39, 2.15	1.0	
	20.0–38.8	6	1.53	0.65, 3.59	1.73	0.55, 5.40
	>38.8	4	1.21	0.43, 3.45	1.65	0.45, 6.09
			p-trend = 0.61		*p*-trend = 0.56	
Lung (code 162)	No exposure	57	1.0			
	<20	9	1.01	0.48, 2.15	1.0	
	20.0–38.8	3	0.54	0.17, 1.75	0.59	0.15, 2.26
	>38.8	15	2.41	1.31, 4.43	3.19	1.28, 7.93
			p-trend = 0.005		*p*-trend = 0.002	
Prostate (code 185)	No exposure	299	1.0			
	<20	56	1.41	1.05, 1.88	1.0	
	20.0–38.8	32	1.28	0.88, 1.85	0.95	0.61, 1.47
	>38.8	26	1.19	0.79, 1.81	0.94	0.58, 1.52
			p-trend = 0.34		*p*-trend = 0 .82	
Melanoma (code 172)	No exposure	31	1.0			
	<20	7	1.67	0.73, 3.87	1.0	
	20.0–38.8	2	0.75	0.18, 3.15	0.44	0.09, 2.22
	>38.8	2	0.71	0.16, 3.04	0.30	0.06, 1.62
			p-trend = 0.59		*p*-trend = 0.21	
Lymphohematopoietic (codes 200–208)	No exposure	67	1.0			
	<20	10	1.17	0.60, 2.29	1.0	
	20.0–38.8	7	1.31	0.60, 2.90	1.16	0.44, 3.07
	>38.8	9	1.84	0.89, 3.82	1.54	0.60, 3.96
			p-trend = 0.094		*p*-trend = 0.37	
Non-Hodgkin's lymphoma (codes 200 and 202)	No exposure	26	1.0			
	<20	6	1.76	0.72, 4.35	1.0	
	20.0–38.8	3	1.36	0.40, 4.56	0.85	0.21, 3.42
	>38.8	2	0.92	0.21, 4.05	0.51	0.10, 2.70
			p-trend = 0.95		*p*-trend = 0.44	
Leukemia (codes 204–208)	No exposure	21	1.0			
	<20	3	1.10	0.32, 3.72	1.0	
	20.0–38.8	4	2.62	0.88, 7.82	2.17	0.48, 9.86
	>38.8	4	3.36	1.08, 10.49	2.93	0.62, 13.90
			p-trend = 0.026		*p*-trend = 0.23	

* ICD-9, *International Classification of Diseases,* Ninth Revision; RR, rate ratio; CI, confidence interval.

† Adjusted for age (<40, 40–49, 50–59, 60–69, 70–79, ≥80 years), smoking (never, pack-years among former smokers and pack-years among current smokers), education, family history of cancer, state of residence, and total days of any pesticide application.

TABLE 3. Rate ratios for selected cancers through December 2002, by intensity-weighted exposure days to diazinon, in male Agricultural Health Study pesticide applicators

Cancer site (ICD-9* classification)	Intensity-weighted no. of exposure days	No. of cases	Referent: nonexposed category		Referent: low-exposed category	
			RR*,†	95% CI*	RR†	95% CI
All neoplasms (codes 140–208)	No exposure	722	1.0			
	Tertile 1	85	1.19	0.95, 1.50	1.0	
	Tertile 2	81	1.09	0.86, 1.38	0.94	0.69, 1.28
	Tertile 3	81	1.28	1.01, 1.63	1.15	0.84, 1.59
			p-trend = 0.05		p-trend = 0.25	
Colorectal (codes 153 and 154)	No exposure	57	1.0			
	Tertile 1	5	1.00	0.40, 2.51	1.0	
	Tertile 2	9	1.76	0.86, 3.60	1.89	0.63, 5.69
	Tertile 3	2	0.53	0.13, 2.23	0.61	0.12, 3.26
			p-trend = 0.55		p-trend = 0.37	
Lung (code 162)	No exposure	57	1.0			
	Tertile 1	6	0.89	0.35, 2.24	1.0	
	Tertile 2	8	1.21	0.57, 2.57	1.47	0.47, 4.57
	Tertile 3	13	1.76	0.92, 3.33	2.45	0.83, 7.22
			p-trend = 0.076		p-trend = 0.093	
Prostate (code 185)	No exposure	299	1.0			
	Tertile 1	43	1.44	1.04, 1.98	1.0	
	Tertile 2	40	1.27	0.91, 1.78	0.91	0.59, 1.41
	Tertile 3	31	1.25	0.85, 1.83	0.97	0.60, 1.56
			p-trend = 0.28		p-trend = 0.98	
Melanoma (code 172)	No exposure	31	1.0			
	Tertile 1	4	1.27	0.44, 3.63	1.0	
	Tertile 2	5	1.53	0.59, 3.99	1.15	0.30, 4.39
	Tertile 3	2	0.62	0.14, 2.67	0.37	0.06, 2.25
			p-trend = 0.57		p-trend = 0.22	
Lymphohematopoietic (codes 200–208)	No exposure	67	1.0			
	Tertile 1	8	1.23	0.59, 2.58	1.0	
	Tertile 2	7	1.05	0.48, 2.30	0.82	0.30, 2.27
	Tertile 3	11	2.01	1.02, 3.94	1.55	0.59, 4.05
			p-trend = 0.049		p-trend = 0.21	
Non-Hodgkin's lymphoma (codes 200 and 202)	No exposure	26	1.0			
	Tertile 1	5	1.94	0.73, 5.09	1.0	
	Tertile 2	2	0.73	0.17, 3.11	0.37	0.07, 1.94
	Tertile 3	4	1.70	0.56, 5.18	0.82	0.20, 3.30
			p-trend = 0.44		p-trend = 0.90	
Leukemia (codes 204–208)	No exposure	21	1.0			
	Tertile 1	2	0.99	0.23, 4.24	1.0	
	Tertile 2	5	2.46	0.91, 6.66	2.41	0.46, 12.63
	Tertile 3	4	2.88	0.92, 9.03	2.77	0.47, 16.26
			p-trend = 0.053		p-trend = 0.38	

* ICD-9, *International Classification of Diseases,* Ninth Revision; RR, rate ratio; CI, confidence interval.

† Adjusted for age ($<$40, 40–49, 50–59, 60–69, 70–79, $\geq$80 years), smoking (never, pack-years among former smokers and pack-years among current smokers), education, family history of cancer, state of residence, and total days of any pesticide application.

TABLE 4. Rate ratios for selected cancers through December 2002, by lifetime and intensity-weighted exposure days to diazinon, in male Agricultural Health Study pesticide applicators*

Cancer site	No. of cases	Referent: nonexposed category		Referent: low-exposed category	
		RR†,‡	95% CI†	RR‡	95% CI
		Lifetime exposure days			
All neoplasms					
No exposure	722	1.0			
<20	106	1.12	0.91, 1.38	1.0	
20.0–38.8	64	1.08	0.83, 1.39	1.00	0.73, 1.37
38.9–108.8	45	1.28	0.93, 1.73	1.23	0.86, 1.77
>108.8	32	1.58	1.10, 2.28	1.54	1.02, 2.33
		p-trend = 0.007		p-trend = 0.029	
Lung					
No exposure	57	1.0			
<20	9	1.02	0.48, 2.16	1.0	
20.0–38.8	3	0.55	0.17, 1.76	0.58	0.15, 2.25
38.9–108.8	7	1.82	0.81, 4.08	2.53	0.87, 7.32
>108.8	8	3.46	1.57, 7.65	4.16	1.47, 11.81
		p-trend = 0.001		p-trend = 0.002	
Prostate					
No exposure	299	1.0			
<20	56	1.41	1.05, 1.88	1.0	
20.0–38.8	32	1.28	0.88, 1.84	0.95	0.61, 1.47
38.9–108.8	16	1.13	0.67, 1.87	0.89	0.50, 1.56
>108.8	10	1.31	0.69, 2.49	1.04	0.52, 2.08
		p-trend = 0.35		p-trend = 0.92	
Lymphohematopoietic					
No exposure	67	1.0			
<20	10	1.17	0.60, 2.29	1.0	
20.0–38.8	7	1.31	0.60, 2.89	1.16	0.44, 3.07
38.9–108.8	6	1.94	0.82, 4.59	1.59	0.56, 4.49
>108.8	3	1.67	0.51, 5.47	1.46	0.38, 5.60
		p-trend = 0.24		p-trend = 0.59	

Table continues

and leukemia with exposure to alachlor (28). To confirm that the results seen in this analysis were not due to exposure to alachlor, we included it in the model, with no change in the results. Other analyses of lung cancer and pesticides in this cohort reported an association with four pesticides (chlorpyrifos, metolachlor, pendimethalin, and carbofuran) in addition to diazinon (22, 29). The observed associations with diazinon were not changed when we adjusted for these pesticides (data not shown).

We examined the importance that timing of exposure may have played in the observed associations by excluding those participants whose exposure to diazinon was the most recent. When we restricted our analyses to those who had used diazinon for the first time prior to the 1990s, we found similar risk estimates (data not shown).

There were nine lung cancer cases among nonsmokers overall and only three lung cancer cases in nonsmokers who reported using diazinon. Thus, we were unable to examine the effect of diazinon use on lung cancer risk among nonsmokers. To further explore potential sources of confounding, we assessed the correlation between smoking and exposure to lifetime days and intensity-weighted days of diazinon use. For both exposure metrics, the correlation was small ($r = 0.02$ for lifetime diazinon days and $r = 0.03$ for intensity-weighted days). The small sample size hindered exploration of consistency with applicator type (commercial vs. private) and state of residence.

DISCUSSION

We did not observe an association between diazinon use and risk of cancer for most sites, including some, such as non-Hodgkin's lymphoma, that had been seen previously in

TABLE 4. Continued

Cancer site	No. of cases	Referent: nonexposed category		Referent: low-exposed category	
		RR‡	95% CI	RR‡	95% CI
Intensity-weighted lifetime exposure days					
All neoplasms					
No exposure	722	1.0			
Tertile 1	85	1.10	0.95, 1.49	1.0	
Tertile 2	81	1.09	0.86, 1.38	0.94	0.69, 1.28
Tertile 3: low	39	1.16	0.84, 1.62	1.03	0.70, 1.52
Tertile 3: high	42	1.41	1.03, 1.95	1.31	0.89, 1.94
		p-trend = 0.033		p-trend = 0.10	
Lung					
No exposure	57	1.0			
Tertile 1	6	0.89	0.35, 2.24	1.0	
Tertile 2	8	1.21	0.57, 2.57	1.46	0.47, 4.56
Tertile 3: low	7	1.97	0.88, 4.45	2.81	0.85, 9.22
Tertile 3: high	6	1.55	0.65, 3.72	2.11	0.61, 7.31
		p-trend = 0.22		p-trend = 0.34	
Prostate					
No exposure	299	1.0			
Tertile 1	43	1.44	1.04, 1.99	1.0	
Tertile 2	40	1.27	0.91, 1.78	0.92	0.59, 1.41
Tertile 3: low	15	1.10	0.65, 1.86	0.83	0.46, 1.52
Tertile 3: high	16	1.47	0.85, 2.40	1.15	0.63, 2.10
		p-trend = 0.20		p-trend = 0.58	
Lymphohematopoietic					
No exposure	67	1.0			
Tertile 1	8	1.24	0.59, 2.59	1.0	
Tertile 2	7	1.05	0.48, 2.31	0.82	0.30, 2.80
Tertile 3: low	5	1.66	0.66, 4.21	1.27	0.40, 3.97
Tertile 3: high	6	2.44	1.02, 5.87	1.97	0.64. 6.08
		p-trend = 0.037		p-trend = 0.14	

* The highest tertile of exposure was split at the median of that tertile.

† RR, rate ratio; CI, confidence interval.

‡ Adjusted for age (<40, 40–49, 50–59, 60–69, 70–79, ≥80 years), smoking (never, pack-years among former smokers and pack-years among current smokers), education, family history of cancer, state of residence, and total days of any pesticide application.

the epidemiologic literature (18, 19). For a few other cancer sites, we did observe an increased risk with exposure to diazinon, including lung cancer, leukemia, and all cancer sites combined when lifetime exposure days was used as the exposure metric and the nonexposed group as the referent.

The observed association with lung cancer was reported previously in this study and elsewhere (22, 23). Another study showed that diazinon produced a dose-response, genotoxic effect on human mucosal cells, suggesting a possible mechanism for carcinogenesis (17). Since lung cancer is highly associated with tobacco use, it is possible that the association observed in this study was due to uncontrolled confounding by smoking. However, we modeled the risk estimates by using a variety of smoking metrics—including never, low, and high exposure; tertiles of exposure for former and current smokers; and continuous pack-years—and the results were unchanged, suggesting that confounding due to smoking probably does not explain the elevated risks of lung cancer. In a previous study of lung cancer from the Agricultural Health Study that was based on fewer years of follow-up and focused on the potential link between multiple pesticides and lung cancer risk, associations were seen with diazinon, as well as chlorpyrifos, metolachlor, and pendimethalin (22). The point estimates for diazinon reported in this analysis did not change when these other three pesticides were included in the model, suggesting that our results were not due to residual confounding by these other pesticides.

In addition to the lifetime exposure days metric, we were able to use a previously published exposure metric that accounts for intensity of exposure as well as duration and frequency (26). When we used this metric, we observed an attenuation of risk estimates for all cancer sites and for lung cancer and leukemia. The association with all cancer remained significant when the nonexposed group was used as the referent, but the associations with lung cancer and leukemia did not. When using the intensity-weighted metric, we observed an increased risk for lymphatic and hematopoietic cancers, which we did not see when using lifetime exposure days.

The intensity-weighting algorithm accounts for differences in exposure that may not be captured by simply examining lifetime days of exposure. However, the algorithm may not be the most appropriate exposure metric for all cancer sites. The intensity-weighting algorithm weighs dermal exposure more heavily than inhalation exposure (26). Absorption of diazinon through human skin has been shown to be in the range of 0.035 $\mu g/cm^2$ (30). For certain cancer sites, such as the lung, inhalation exposure may be more important than dermal absorption for carcinogenesis. While the estimates of risk for lung cancer and leukemia were not significantly elevated for intensity-weighted diazinon exposure, there did appear to be increasing monotonic trends for both cancers. This finding, coupled with the associations observed when lifetime exposure days were used, lends support to a potential association between diazinon and these two cancers.

We did not find any association between non-Hodgkin's lymphoma and diazinon exposure when either exposure metric was used. However, these estimates are based on a small number of exposed cases ($n = 12$) and suggest that a potential relation should be explored in more detail as more incident cases of non-Hodgkin's lymphoma accumulate in this cohort.

We found no association between diazinon and prostate cancer, which is consistent with a previous report from the Agricultural Health Study (31).

When the intensity-weighted metric was used, we observed an association with lymphohematopoietic cancer. Although this finding is suggestive of an association, the interpretation is unclear since the lymphatic and hematopoietic cancer category aggregates many cancer types. Aggregation is a form of disease misclassification and is not likely to cause a false-positive association. For reasons of small sample size, we were unable to examine the constituent sites individually.

Because the usage profile for diazinon differed between the two participating states, we examined the consistency of the observed associations by stratifying on state of residence. It appeared that the associations with diazinon use and risk of lung cancer and leukemia were somewhat stronger in Iowa than in North Carolina, although we found no effect modification by state for any cancer site. The numbers were small, however, and underpowered to detect such an effect.

As a class, organophosphates inhibit cholinesterase enzymes, which leads to accumulation of acetylcholine and disturbs transmission across cholinergic synapses (9), making them effective insecticides (12). A variety of immunotoxic and genotoxic responses have been noted in animal and human studies after exposure to organophosphates, suggesting mechanistic routes that could lead to the development of cancer (9, 32). However, at this time, the EPA has classified diazinon as ''not likely a human carcinogen.''

One of the main strengths of this study is its prospective design, allowing for definitive assessment of temporality between exposure and disease occurrence. Another strength is the extensive and detailed information on both duration and intensity of exposure to pesticides. Cases were identified through state cancer registries, resulting in population-based ascertainment of all incident cancers.

A limitation of this study is the relatively small number of exposed incident cases associated with many of the cancer sites despite there being close to 5,000 pesticide applicators who had used diazinon. These small numbers led to some unstable risk estimates and the inability to explore potential effect modification or consistency within subgroups (i.e., applicator type and state of residence). However, this is the largest known study of pesticide applicators to date. The prospective nature of this study will allow further analysis to be conducted as the cohort is followed and more incident cancers occur. Another limitation is that while diazinon was first licensed for use in 1956 (3), the formulation may have changed over time. Using an exposure measurement that considers only duration and intensity does not account for temporal variability in exposure. Finally, it should be noted that the study participants are exposed to numerous chemicals in addition to diazinon. We attempted to minimize confounding from these exposures by examining their effect using a variety of metrics and by adjusting for the possible effects of pesticides, whose use is correlated with that of diazinon. However, it is possible that confounding due to the other exposures could have occurred and biased our results. It is also possible that any association we observed is due to random variability within the data. Given the consistency of the results across referent groups and exposure metrics for lung cancer and leukemia, this explanation seems less likely, however.

This study is one of only a few to examine the effects of diazinon exposure and risk of cancer. We found evidence of an association of lung cancer and leukemia risk with increasing lifetime exposure days to diazinon. While approaching statistical significance, these observed associations were not significantly elevated when the intensity-weighted exposure algorithm was used. As more cases of cancer accrue in the cohort among those exposed to diazinon, analyses that further explore and refine potential associations will be performed. Subsequent analyses in this cohort and others should provide more information about the potential association between diazinon exposure and cancer.

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